Parasitism and Location of Sugarcane Borer (Lepidoptera: Pyralidae) by *Cotesia flavipes* (Hymenoptera: Braconidae) on Transgenic and Conventional Sugarcane

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ABSTRACT   Location and parasitism of *Diatraea saccharalis* (F.) by *Cotesia flavipes* (Cameron) were compared between hosts fed either conventional or transgenic sugarcane expressing *Galanthus nivalis* L. agglutinin (GNA) under choice and no-choice conditions. In olfactometer experiments, females of *C. flavipes* randomly visited the different odor zones available but spent significantly more time in odor zones corresponding to plants damaged by *D. saccharalis* versus blank (control) odor zones. However, they spent similar amounts of time in odor zones corresponding to damaged transgenic and conventional sugarcane. Laboratory and field-cage experiments showed that *C. flavipes* equally parasitized *D. saccharalis* feeding on transgenic and conventional sugarcane plants. Moreover, sex ratio and brood size of *C. flavipes* were similar on hosts fed transgenic or conventional sugarcane. The results of this study suggest that transgenic sugarcane expressing GNA would not significantly affect host location and parasitism of *D. saccharalis* by *C. flavipes* in the field.

KEY WORDS   *Diatraea saccharalis*, snowdrop lectin, GNA, biological control, nontarget effects, sugarcane

SUGARCANE BORER, *Diatraea saccharalis* (F.), was for many years the main insect pest of sugarcane in south Texas. However, its populations declined substantially after successful colonization in the mid-1970s of the larval parasitoid *Cotesia flavipes* (Cameron) and currently represent ≈5% of stalk borers recovered from sugarcane fields (Legaspi et al. 1997, Meagher et al. 1998). At present, Mexican rice borer, *Eoreuma loftini* (Dyar), is the key insect pest of sugarcane in south Texas (Legaspi et al. 1999). Transgenic sugarcane expressing *Galanthus nivalis* L. agglutinin (GNA) at ≈0.9% in leaves and stem tissue was developed to target Mexican rice borer because effective control methods against this pest are not currently available (Irvine and Mirkov 1997; Legaspi et al. 1997). Although the mode of action of GNA in insects is not fully understood, it has been shown to interfere with nutrient uptake by binding mannose in the midgut and thus affecting development (Powell et al. 1998, Down et al. 2000).

Recent laboratory and field studies showed that GNA-expressing transgenic sugarcane negatively affected various fitness parameters of and damage levels caused by Mexican rice borer, whereas at the same time, the effects on sugarcane borer were variable from slightly negative, to nil, and positive (Sétamou et al. 2002a). These results raised concerns that biological control of sugarcane borer could be compromised if GNA expressed in the transgenic sugarcane had significant host-mediated negative effects on the fitness of *C. flavipes.* Thus, the present and prior studies sought to uncover any negative effects of transgenic sugarcane expressing GNA on the fitness and biological control performance of *C. flavipes* (Sétamou et al. 2002b). Few studies have investigated the effects of GNA delivered either via transgenic plants or artificial diet on natural enemies. Lethal effects of GNA were not recorded for the twospotted lady beetle, *Adalia bipunctata* Linnaeus, a predator of the green peach aphid *Myzus persicae* (Sulzer) (Birch et al. 1999, Down et al. 2000). Similarly, direct detrimental effects of GNA on the fitness parameters of the potato aphid parasitoid *Aphelinus abdominalis* Dalman were not recorded, although the lectin indirectly reduced parasitoid size and proportion of females in the progeny (Couty et al. 2001). In another study, Bell et al. (1999) failed to find adverse effects of GNA on the fitness and parasitism success of the parasitoid *Eulophus pennicornis* Nees. In a recent laboratory study, small negative effects of GNA-expressing sugarcane were recorded on the development of *C. flavipes* parasitizing sugarcane borer (Sétamou et al. 2002b). Specifically, slight reductions in adult emergence rates and proportion of female offspring were evident in *C. flavipes* developing on hosts fed GNA-sugarcane relative to

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those developing on hosts fed conventional sugarcane. These findings raised additional concern over the possibility that biological control of sugarcane borer by *C. flavipes* might be disrupted following introduction of GNA-sugarcane. Therefore, the goal of this study was to assess whether a number of host plant, host, and parasitoid attributes important for biological control of sugarcane borer by *C. flavipes* are affected by GNA-sugarcane.

The current study specifically addresses several questions. (1) Are females of *C. flavipes* differentially attracted to GNA- and conventional sugarcane plants damaged by sugarcane borer? (2) Under no-choice conditions, are sugarcane borers fed GNA-sugarcane equally acceptable and suitable hosts as those fed conventional sugarcane? (3) Under choice conditions, are sugarcane borers fed GNA-sugarcane equally acceptable and suitable hosts as those fed conventional sugarcane? And, (4) under field-cage conditions, are females of *C. flavipes* reared on hosts fed GNA-sugarcane equally capable of finding and parasitizing hosts as those reared on hosts fed conventional sugarcane? Addressing this latter question required the use of field-cages and artificial infestations of sugarcane borer because current levels of this pest in south Texas sugarcane are too low to permit a meaningful evaluation (Legaspi et al. 1997, Meagher et al. 1998).

Materials and Methods

Insects. All *C. flavipes* and sugarcane borer larvae used in the experiments were obtained from laboratory cultures maintained at the Texas Agricultural Experiment Station, Weslaco. The rearing methodology for *C. flavipes* and sugarcane borer was described in detail earlier (Sétamou et al. 2002a, 2000b).

Plants. The sugarcane variety CP65–357 was used for all tests. Transgenic line 83 expressing GNA (Irvine and Mirkov 1997) and a nontransformed (conventional) line were planted at the Texas Agricultural Experiment Station, Weslaco, in November 1999. Plants were grown under standard farming practices and were flood-irrigated once a month.

Plants used for the olfactometer tests were grown in the greenhouse in 7.56-liter pots filled with soil (Scotts Metro Mix # 300, Marshville, OH). Ten-cm stalk cuttings with at least one node were collected from 8 mo-old field planted canes and transplanted in the pots. The pots were watered weekly until plants were used in the tests.

Olfactometer Tests. A four-arm olfactometer built of four perspex crescents (90° arc, 27 cm diam), similar to that of Vet et al. (1983) was used in this study. A flask filled with distilled water was used to maintain air humidity at 80–90% RH. Temperature was maintained at 22–25°C. Airflow was set at 20 cm/s in each of the four arms. All tests were conducted in the mornings between 800 and 1100 hours. Two odor sources, sugarcane borer-damaged transgenic and conventional sugarcane, and two blank controls (i.e., no odor) were tested simultaneously.

Two month-old transgenic and conventional sugarcane plants were individually infested with five fourth-instar sugarcane borers reared on artificial diet based on transgenic or conventional sugarcane tissue, respectively. The larvae were allowed to establish and feed on the plants for 48 h before the experiment. Ten-cm damaged stalk cuttings of transgenic or conventional sugarcane were taken from the infested plants. The damaged cuttings were split longitudinally to remove all feeding borers, and then placed with frass in glass flasks at one end of the olfactometer as an odor source. In each test, damaged cuttings were used as odor sources for two opposing olfactometer arms, and the remaining two opposing arms corresponded to blank controls. Individual females of *C. flavipes* were released in the center of the olfactometer arena, and their activity was monitored for 10 min. The numbers of visits and time spent in each odor zone were recorded. In addition, a transverse line was drawn at 5 cm from the end of each olfactometer arm, and trespass of this line by a female was scored as its "first choice." A total of 100 females were tested in this manner. The olfactometer was cleaned with 70% alcohol, and odor sources were replaced and rotated after testing 25 females.

Parasitism Under No-Choice Conditions in the Laboratory. Ten-cm stalk cuttings of transgenic and conventional sugarcane were collected from 8 mo-old field-planted sugarcane. The cuttings were immediately disinfected with 0.5% bleach solution for 5 min and rinsed twice with distilled water. Five cuttings from either transgenic or conventional sugarcane were placed individually in an oblique way in clear plastic containers (10 × 10 × 10 cm) covered with a ventilated lid. Each cutting was infested with five fourth instar sugarcane borers reared on either transgenic or conventional sugarcane-based diet for the corresponding treatment (Sétamou et al. 2002a). These larvae were allowed to establish within the stalk pieces for 24 h, after which two pairs of 24 h-old *C. flavipes* were released in each container. Thus, the experimental set-up consisted of five containers per conventional and transgenic sugarcane, each container with one cutting, five sugarcane borers, and two pairs of *C. flavipes*. The containers were placed in a tray and kept at 25 ± 1°C, 60–70% RH, and a 14:10 (L:D) photoperiod. Under these conditions, females of *C. flavipes* live on average for 48 h (Sétamou et al. 2002b). One week after parasitoid release, the stalk cuttings were split longitudinally to recover sugarcane borer larvae. Recovered larvae were individually reared on artificial diet until formation of parasitoid cocoons or moth pupae. Parasitoid cocoons were incubated until emergence of adult parasitoids. Upon emergence, adult parasitoids were killed and stored in a deep freezer until they were scored. The experiment was repeated twice, with a 1-wk intermission. For each experiment, the percentages of sugarcane borers recovered and parasitized, and the numbers of parasitoid adults (or brood size) emerging per host and their sex ratio were scored for each of the sugarcane lines.

Parasitism Under Choice Conditions in the Laboratory. The experimental procedure used in this experiment was similar to the no-choice test described above with the exceptions noted here. In this test, two stalk cuttings, one each of conventional and transgenic sugarcane, were placed in each of 10 containers (i.e., 2 cuttings/container). A small incision was made at one end of the transgenic cuttings to distinguish from the conventional cuttings. Each of five containers received 10 larvae reared on diet based on either conventional or transgenic sugarcane tissue, for a total of 50 larvae for each of the sugarcane lines. Four pairs of *C. flavipes* were released in the containers after 24 h. The stalk cuttings were dissected 1 wk after parasitoid release, and borers were transferred to artificial diet for incubation, at which time the numbers of borers recovered from each sugarcane line were recorded. Percentages of recovery and parasitism of sugarcane borer as well as the numbers of parasitoid adults (brood size) emerging per host and their sex ratio (% males) were scored. This test was also repeated twice with a 1-wk intermission.

Field-Cage Experiments. Experimental plots were planted in a randomized block design with four blocks of two plots each. The experimental plots consisted of 15 rows; rows were 5 m long and were separated by a 0.6-m alley. The between-plot distance was 3 m within each block, and blocks were located five m apart from each other. Walk-in cages (2 × 2 by 2.5 m) were randomly placed in each plot in the field 10 mo. after planting. The field-cages were constructed of Nitex nylon mesh (363 μm mesh size) (Forestry Suppliers, Jackson, MS) and a metal frame, with a 1-m long zippered opening on one side. Each cage contained 80–100 sugarcane plant stands. Ten plants in each cage were randomly selected and artificially infested with two fourth-instar *D. saccharalis* reared on diet based on either conventional or transgenic sugarcane. Two holes were drilled in each stalk, one at 0.5 m above the ground and the other at 1.5 m, and one larva was placed inside each hole. Holes were plugged with glass vials to prevent escape of larvae. Two days after infestation, the glass vials were removed, and 10 pairs of 1 d-old *C. flavipes* were released in the cages. The field-cages containing transgenic sugarcane received *C. flavipes* from hosts reared on transgenic sugarcane-based diet, whereas the conventional sugarcane received *C. flavipes* from hosts reared on conventional sugarcane-based diet (Sétamou et al. 2002a, 2000b). Infested plants were cut 1 wk after parasitoid release and dissected to recover sugarcane borers. Recovered sugarcane borers were individually reared on artificial diet until formation of parasitoid cocoons or moth pupae. The proportions of parasitized sugarcane borer larvae, and numbers and sex ratio of parasitoid adults per host were scored.

Data Analysis. Friedman's one-way analysis of variance (ANOVA) by ranks, based on number of visits, number of first choices and time spent per odor zone, was used to test for odor zone preference by *C. flavipes* females in the olfactometer tests (Zar 1999). Means
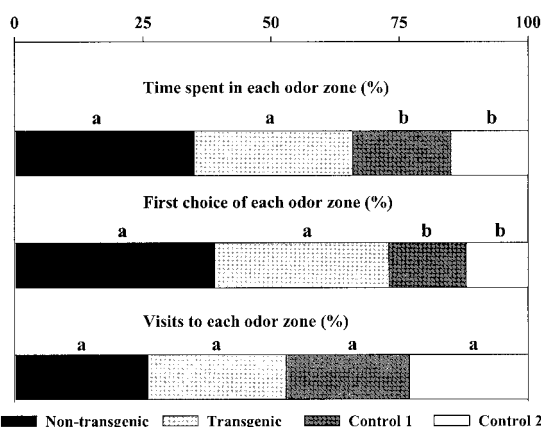

Fig. 1. Responses of *Cotesia flavipes* females to the odors of GNA-expressing transgenic and conventional sugarcane tissue damaged by *Diatraea saccharalis* larvae and two blank (control) odors in a four-arm olfactometer. Different letters above each bar segment indicate significant differences within bar (Time spent, $F = 3.41$, $P = 0.0002$, df = 3, 235; Visits, $F = 1.85$, $P = 0.14$, df = 3, 235; First choice, $\chi^2 = 10.95$, $P = 0.012$, df = 3).

were separated as warranted using the Student Newman-Keuls procedure for ranked data (Zar 1999).

Variance homogeneity tests were performed to compare data from the two tests in each of the laboratory choice and no-choice experiments (Zar 1999). Data from the two tests were pooled before further analyses whenever variances were homogeneous. Log-likelihood ratio tests were used to compare the percentages of sugarcane borer larvae recovered and parasitized, and parasitoid sex ratios between the conventional and transgenic sugarcane lines in the laboratory and field-cage tests (Zar 1999). Mean brood sizes per larva recorded in both sugarcane lines were compared via *t*-tests (Zar 1999).

Results

Olfactometer Tests. *Cotesia flavipes* females spent significantly more time in ($P = 0.018$) and more frequently selected as their first choice ($P = 0.012$) the odor zones containing damaged tissue of either transgenic or conventional sugarcane relative to the two blank odor zones (Fig. 1). However, significant differences were not evident between the odor zones corresponding to damaged transgenic or conventional tissues (Fig 1). Moreover, significant differences were not evident among the frequency of visits to each of the four odor zones ($P = 0.14$), suggesting that *C. flavipes* females randomly visited each of the odor zones before selecting any given zone (Fig. 1).

Parasitism Under No-Choice Conditions. Recovery levels of sugarcane borer larvae under no-choice conditions were relatively high ($\approx70\%$) for transgenic and conventional sugarcane (Fig. 2a). Moreover, recovery levels were similar for transgenic and conventional sugarcane ($P > 0.25$). Parasitism rates of sugarcane borer larvae were low ($\approx25\%$) in relation to recovery
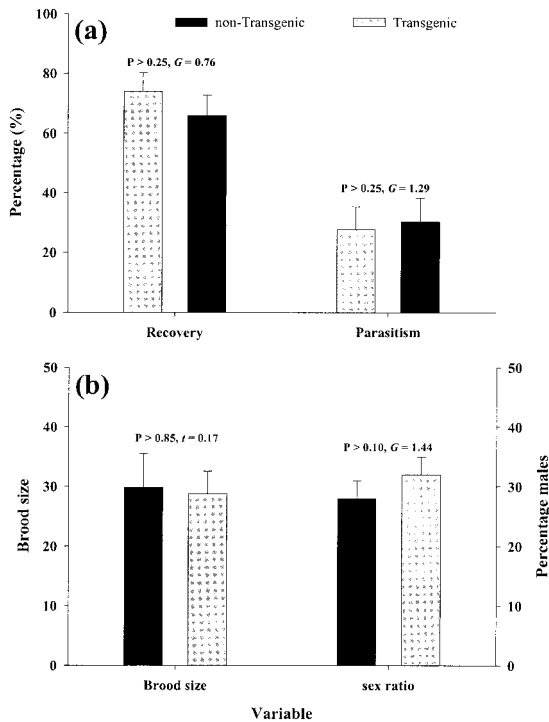
Fig. 2. (a) Recovery of *Diatraea saccharalis* larvae and parasitism by *Cotesia flavipes*, and (b) brood size and sex ratio of *C. flavipes* under no-choice conditions on transgenic and conventional sugarcane in the laboratory. Data shown correspond to combined results of two trials (see text for explanation).
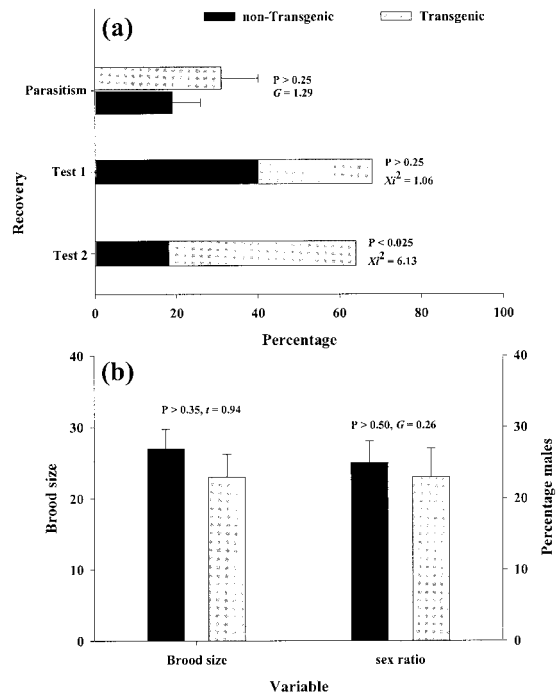

Fig. 3. (a) Recovery of *Diatraea saccharalis* larvae and parasitism by *Cotesia flavipes*, and (b) brood size and sex ratio of *C. flavipes* under choice conditions on transgenic and conventional sugarcane in the laboratory. Except for "Recovery," data shown correspond to combined results of two trials (see text for explanation).

rates for transgenic and conventional sugarcane (Fig. 2a). In addition, parasitism rates were similar for transgenic and conventional sugarcane ($P > 0.25$). *Cotesia flavipes* brood sizes (numbers of adults emerging per host) ($P > 0.85$) and offspring sex ratios (% males) ($P > 0.10$) were similar on hosts fed transgenic or conventional sugarcane (Fig. 2b).

Parasitism Under Choice Conditions. Recovery levels of sugarcane borer larvae under choice conditions were relatively high ($\approx 70\%$) for the first and second tests (Fig. 3a). However, significantly more than expected sugarcane borer larvae were recovered from transgenic sugarcane relative to conventional sugarcane in the first test ($P < 0.025$), whereas similar numbers were recovered from transgenic and conventional sugarcane in the second test ($P > 0.25$). Parasitism rates of sugarcane borer larvae were low ($\approx 25\%$) in relation to recovery rates for transgenic and conventional sugarcane (Fig. 3a). In addition, parasitism rates were similar for transgenic and conventional sugarcane ($P > 0.25$). *Cotesia flavipes* brood sizes ($P > 0.35$) and offspring sex ratios ($P > 0.50$) were similar on hosts fed transgenic or conventional sugarcane (Fig. 3b).

Parasitism Under Field-Cage Conditions. Recovery levels of sugarcane borer larvae under field-cage conditions ($\approx 40\%$) were lower than the levels observed

under choice and no-choice conditions in the laboratory (Fig. 4a). Significantly more sugarcane borer larvae were recovered from conventional sugarcane relative to transgenic sugarcane ($P < 0.01$). However, parasitism rates of sugarcane borer larvae were variable in relation to recovery rates (Fig. 4b). The parasitism rate of sugarcane borer larvae was significantly higher on transgenic relative to conventional sugarcane ($P < 0.01$). *Cotesia flavipes* brood sizes ($P > 0.80$) and offspring sex ratios ($P > 0.50$) were similar on hosts fed transgenic or conventional sugarcane (Fig. 4b).

Discussion

The results of the olfactometer experiment showed that damaged transgenic and conventional sugarcane were similarly attractive to females of *C. flavipes*, whereas both were more attractive than a blank control odor. These results also revealed that females of *C. flavipes* are attracted to sugarcane plants infested with sugarcane borer and suggest that plant- and host-derived cues from conventional and GNA-sugarcane are similarly attractive to females of *C. flavipes* searching for sugarcane borer hosts. The attraction of *C. flavipes* to plants damaged by sugarcane borer observed in this study is in accordance with the results of previous studies showing that this parasitoid is at-
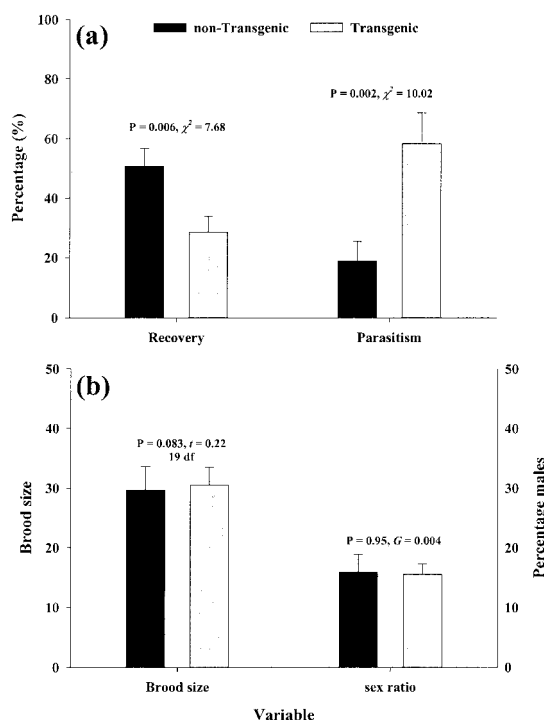
Fig. 4. (a) Recovery of *Diatraea saccharalis* larvae and parasitism by *Cotesia flavipes*, and (b) brood size and sex ratio of *C. flavipes* in field-cages in transgenic and conventional sugarcane plots.

tracted to plants infested and uninfested with hosts (Ngi-Song and Overholt 1997, Potting et al. 1997). However, the similar degrees of attractiveness of transgenic and conventional sugarcane plants to females of *C. flavipes* suggest that the presence of GNA in sugarcane plants will not affect the host location process of *C. flavipes.* Similarly, differences were not found in the attractiveness of herbivore-damaged *Bt* transgenic and conventional oilseed rape plants to the braconid *Cotesia plutellae* (Kurdjimov) (Schuler et al. 1999).

In the choice and no-choice experiments in the laboratory, the recovery of sugarcane borer larvae from transgenic sugarcane was either similar to or higher than that recorded from conventional sugarcane suggesting that GNA did not adversely affect the establishment of sugarcane borer in their feeding sites. The two experiments also showed that sugarcane borer larvae feeding on either transgenic or conventional sugarcane were equally attractive to and accepted for parasitism by *C. flavipes.* Moreover, both experiments suggest that sugarcane borer larvae feeding on either transgenic or conventional sugarcane are similarly suitable hosts for *C. flavipes.*

Similar to the laboratory tests, the results of the field experiment suggested that sugarcane borer larvae feeding on either transgenic or conventional sugarcane were readily located and equally suitable hosts for *C. flavipes.* However, parasitism rates were higher

on the transgenic sugarcane compared with the conventional sugarcane. Host location by parasitoids is an important step for successful parasitization and is mediated by host plant, host, and host-related cues. Thus, the higher rate of parasitism in the transgenic sugarcane may be explained by the larger size that sugarcane borer larvae attain when feeding on transgenic versus conventional sugarcane (Sétamou et al. 2002a), and by higher damage and establishment levels of larvae in the transgenic sugarcane plots relative to the conventional plots. Parasitized sugarcane borer larvae occurred more frequently in sugarcane plants with "high" (73% of parasitized larvae) versus "low" (26%) damage scores ($\chi^2 = 11.3$, df = 1, $P < 0.001$, data not shown), and transgenic plants (55% of plants) received "high" damage scores (likely indicating successful establishment of sugarcane borer larvae) more frequently than conventional plants (36%), although the difference was marginally significant ($G = 3.50$, df = 1, $P = 0.07$, data not shown). Consequently, females of *C. flavipes* may have more easily located and parasitized sugarcane borer larvae within transgenic plants relative to those in conventional plants because of greater plant damage, frass-derived cues, and wider galleries for accessing hosts.

Larvae of *C. flavipes* are endophagous and feed on host tissues and fluids (Smith et al. 1993). In western blot analyses, GNA was detected in sugarcane borer larvae reared on diet containing transgenic sugarcane tissue (MS, unpublished data). In addition, prior studies showed that GNA ingested by larvae of *Lacanobia oleracea* (L.) accumulates in the gut and is present in the hemolymph and thus is readily available to parasitoids feeding on these larvae (Fitches et al. 1997, Fitches and Gatehouse 1998). Thus, larvae of *C. flavipes* are likely subject to any potential direct effects of GNA in host tissues. Sétamou et al. (2002b) showed that some fitness parameters of *C. flavipes* were negatively, although marginally, affected when developing on sugarcane borer larvae reared on diet containing GNA-sugarcane tissue. In contrast, negative direct effects of GNA on *C. flavipes* were not evident in this study.

These conflicting results are significant given that *C. flavipes* were likely exposed to greater concentrations of GNA in this study because hosts were fed transgenic sugarcane tissue (≈0.9% GNA), whereas hosts in the Sétamou et al. (2002b) study were fed artificial diet containing transgenic sugarcane tissue (≈0.5% GNA). However, Sétamou et al. (2002b) used 18 d-old sugarcane borer larvae as hosts in their study, whereas younger, fourth instars (≈10 d old) were used at the beginning of all the experiments in this study. In addition, sugarcane borer larvae developing on diet containing transgenic sugarcane grow faster, likely ingest more GNA, and are physiologically older at the time of parasitization than those on diet containing conventional sugarcane (Sétamou et al. 2002a). Thus, the differential effects of GNA on development of *C. flavipes* could be due to differences in host quality at parasitization, with fewer host resources for parasitoid development and/or stronger host immune responses

occurring in the earler study by Sétamou et al. (2002b) relative to this study. Wiedenmann et al. (1992) showed that although third to sixth instars were acceptable and suitable hosts for *C. flavipes*, sixth instars were the least suitable of these. Similarly, Tanwar and Varma (1996) reported that third to fifth instars of *Chilo auricilius* Dudgeon were more suitable than older larvae for development of *C. flavipes.* Alternatively, qualitative differences between transgenic sugarcane and diet containing transgenic sugarcane may have led to quantitative differences in GNA uptake by host larvae leading to differential effects on developing parasitoids. However, this explanation remains to be examined experimentally.

Studies on potential side effects of transgenic insecticidal crop cultivars on natural enemies and other nontarget organisms are increasingly attracting attention (Bell et al. 1999, Birch et al. 1999, Losey et al. 1999, Schuler et al. 1999, Hilbeck et al. 2000, Jesse and Obrycki 2000, Wraight et al. 2000, Couty et al. 2001). Such studies are necessary if these cultivars are to become widespread integrated pest management (IPM) tactics. A number of researchers have hypothesized that transgene-derived proteins may be directly or indirectly toxic to parasitoids and that they may affect the host location process of parasitoids (Schuler et al. 1999). However, the results of these laboratory and field-cage studies show that parasitism, suitability, and host location by *C. flavipes* were not significantly affected by GNA present in the transgenic sugarcane. Although some marginal and sublethal effects were observed at the individual level in a previous study (Sétamou et al. 2002b), data available to date suggest that GNA-sugarcane will not strongly affect the behavior, development, and biological control potential of *C. flavipes* in the field.

Acknowledgments

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